Remarks

1 – 4. Applicants acknowledge the Examiner's issuance of a final rejection. Applicants request an opportunity to have a timely interview if the Examiner remains unpersuaded as to the patentability of one or more claims as currently amended and in view of the following remarks.

5. A new oath/declaration is included with this paper.

6. a) New matter has been removed from Claim 1 through the present amendment.

b) Two additional amendments of the specification are entered to correct typographical errors. The first, in the paragraph beginning on line 18, page 16 of the specification contains no new matter as it is clear from the outset of the paragraph and the experimental details presented in the referenced Table 4 that the substitutions under discussion are T117R and G188L and not T117R and G118L. The second, in the paragraph beginning on line 28, page 32 also contains no new matter as the remainder of the specification clearly discusses residue P179 and not P170 which was typed in error.

7. Rejection under 35 USC §102 (b) and (e).

The Examiner has again rejected the Claims in the case stating that Cahoon et al. [PNAS 94:4872-4877, May 1977] anticipates the Claims, and noting that

> "specific mutants at positions 114, 117, 118, 179, 181 & 188 are also taught (see the entire document, especially abstract, Fig. 3, and page 4875-column 2). The reference also teaches specific positions which can be **replaced by any amino acid** or which can be used for making two or more amino acid substitutions in the castor Δ^9-18:0-ACP desaturase and having increased activity towards fatty acids having fewer that (*sic*) 18 carbons [see column 5, lines 50-60]. (emphasis and underlining added)

Again the Applicants request reconsideration and withdrawal of this basis for rejection for the following reasons.

Applicants acknowledge that the reference identifies candidate amino acids that if substituted would be likely to modify the activity of the desaturase. In fact the reference identifies more candidate positions than the positions cited by the Examiner, specifically identifying amino acids 114, 115, 117, 118, 179, 181, 188 and 189. The reference does not therefore anticipate the present invention as it is unclear from the reference which of the 8 amino acid positions should be substituted. Further, there is no suggestion in the reference and certainly none is implied that any of the 8 residues could be **replaced by any amino acid**. In fact it would be readily apparent to one of average skill that it is highly unlikely that substitution with **any** amino acid at any of the positions would produce an enzyme with desirable substrate specficity.

The Examiner further suggests that Figure 3 provides sufficient information about what substitutions to make in the Δ^9-18:0-ACP desaturase. However, the Figure caption reads in part "Residues that have been replaced in mutants of the Δ^6-16:0-ACP desaturase are indicated in cyan, and mutant L118F/P179I is shown in magenta". Thus the Figure shows substitutions that were made in the Δ^6-16:0-ACP desaturase (summarized in Table 1 of the

reference) and shows the only mutant of the castor Δ^9-18:0-ACP desaturase (L118F and P179I) that was described in the referenced article. Thus this aspect of the reference does not anticipate the present invention.

As an indication as to how the reference, rather than anticipating the present invention, actually directs away from the present invention, the Applicants respectfully draw the Examiner's attention to page 4876, left column beginning in the section headed **Rational Design of Acyl-ACP Desaturase Activity.** The first sentence of that paragraph states: "Residues described above and shown in Fig. 4 that line the lower portion of the active site represent potential targets for the rational design of acyl-ACP desaturase activities with respect to fatty acid chain-length specificity." Figure 4 of page 4876 compares 8 candidate amino acids residues found in four (4) different desaturases. In making this comparison, the authors were suggesting that substitution of the candidate amino acids of the Δ^9-18:0 ACP desaturase with amino acids found in cognate positions in a Δ^6-16:0-ACP desaturase, a Δ^9-14:0-ACP desaturase or a Δ^4-14:0-ACP desaturase might produce a mutant 18:0 desaturase with an increased reactivity toward substrates having fewer than 18 carbons. The following table summarizes the comparison and demonstrates how the suggestion and guidance provided therein directs away from the present invention.

Amino Acid Position	Δ^9-18:0 desaturase	Δ^6-16:0 desaturase	Δ^9-14:0 desaturase	Δ^4-16:0 desaturase	Substitution(s) of the Present Invention
114	Met	Met	Leu	Met	Ala or Thr
115	Leu/Ile	Leu	Val	Leu	*none*
117	Thr	Thr	Arg	Arg	Arg
118	Leu	Thr	Pro	Cys	Gly or Ala
179	Pro	Pro	Ile	Thr	Val or Leu
181	Thr	Ala	Thr	Thr	Val or Ser
188	Gly	Ala	Leu	Gly	Leu
189	Phe	Tyr	Phe	Phe	*none*

Only in the case of the substitution of Arg for Thr at position 117 was the suggestion of the reference directed toward a substitution that is found in all mutants of the present invention. Clearly the remainder of the guidance directs away from the claimed mutant desaturases of the present invention and therefore the reference cannot anticipate the Claims.

8. Rejection under 35 U.S.C. §102 as being anticipated by US Patent No. 5,705,391 (Cahoon et al, Jan. 6, 1998). The Applicants respectfully request withdrawal of this rejection and reiterate the above remarks. This reference clearly shows how to alter the activity of a Δ^6-16:0-ACP desaturase so that it has increased activity with substrates having 18 carbon atoms but does not specifically anticipate which specific amino acid substitutions would increase the substrate specificity of an 18:0 desaturase toward fatty acids having fewer than 18 carbons. The reference identifies amino acid residues that are likely to play an important role in substrate specificity, again identifying 8 candidate amino acids as in the Cahoon, et al. PNAS reference. However it is clear from this reference that there is no expectation that these candidate amino acids could be replaced **by any other amino acid**. The Applicants respectfully direct the Examiner's attention to column 4, lines 13 – 44 wherein the inventors enunciate the care that must be exercised in making a substitution so as not to destroy the enzyme activity. Included therein are the following remarks on this topic:

> "**Care must be exercised in selecting a residue to be substituted for an existing contact residue in the substrate binding channel of a wild type acyl-ACP desaturase.** It is generally important in initial studies, for example, to select residues for substitution which do not differ radically with respect to side chain size or charge." (lines 15 – 21) (emphasis added)
>
> and

> "Thus, it is the knowledge of the identity of the contact residues within an acyl-ACP desaturase that allow one skilled in the art to make modifications to the enzyme that can alter chain length **and** double bond positional specificity of the enzyme **without inhibiting its ability to carry out enzyme catalysis**." (lines 39-44) (emphasis added)

Since the reference does not direct one to make the specific substitutions in the six (6) amino acid residues of the presently claimed invention, the reference does not anticipate the Claims.

9, 10. Rejection under 35 USC 102(e) as being anticipated by U.S. Patent No. 5,888,790, particularly Claims 10-12; and rejection under the judicially created doctrine of double patenting claims 7-20 of US Patent No. 5,888,790, claims 7-20.

The Applicants respectfully request withdrawal of these grounds for rejection. The mutant desaturases of the presently claimed invention are specific species encompassed within the genus of the cited claims 10-12. As the reference provides little or no guidance as to which amino acids could be effectively substituted for the wild type residues, and in fact states the care and caution that must be exercised in making substitutions (Column 4, lines 26-32 and lines 50-55), it is difficult to understand how such a genus could anticipate the specific mutants of the presently claimed invention.

With respect to double patenting, clearly the mutants of the presently claimed invention are not chimeric desaturases of claims 14 through 20 and therefore allowance of the claims pending in the present case would not unduly extend the term of those claims.

With respect to claims 7 through 12, as noted above, the mutant desaturases of the present invention are specific species encompassed within the genus of the cited claims.

Because the owner of the cited patent (Brookhaven Science Associates, LLC.) is, in part, the owner of the presently claimed invention and because the inventors of the presently claimed invention have a duty to assign to Brookhaven Science Associates, LLC., the Applicants request that the Examiner rescind these grounds for rejection upon review and acceptance of the Applicant's terminal disclaimer, the petition for which is included with this paper.

11. Rejection of Claims 1, 3-5 under 35 USC 112 (second paragraph) as being indefinite.

The Applicant's have complied with the Examiner's suggestion that a "positive expression clearly defining the extent of increase will overcome this rejection" in the current amendment of Claim 1. This aspect of the amendment of Claim 1 is supported by the specification and therefore contains no new matter. The specification outlines the boundaries of "substantial increase" as being an increase that allows survival of the transformed unsaturated fatty acid auxotroph host cells under selective conditions (i.e., media lacking unsaturated fatty acid supplements) and also defines the extent of increase this represents. As put forth on page 11 (lines 3 through 18, in particular lines 6 through 18):

> A mutant fatty acid desaturase identified by the above selection assay has a substantial increase in the activity towards fatty acid substrates with chains containing fewer than 18 carbons, relative to the original desaturase. A substantial increase in substrate specificity with respect to the original desaturase is one that produces sufficient accumulation of unsaturated fatty acids, which results from desaturation by the mutant desaturase, within an unsaturated fatty acid auxotroph host organism so as to support growth and proliferation of the host organism. Substantial increase in activity sufficient to support growth of the auxotroph host is at least three-fold higher than that of the non-mutagenized precursor desaturase. In a preferred embodiment, the increase in activity of the mutant desaturase is at least ten-fold higher than the non-mutagenized precursor desaturase. (emphasis added)

Thus, the amended Claim 1 contains no new matter.

Additional Remarks

New Claim 61 has been added. Claim 61 contains no new matter.

The additional claims currently amended in this paper contain no new matter.

In light of the above Amendments and Remarks, applicants respectfully submit that the instant application is now in condition for allowance and solicit a timely notice of allowance. If the Examiner finds the above amendments and remarks unpersuasive, as requested in items 1-4 of this paper, the Applicants respectfully request an interview with the Examiner so as to assist the Examiner in gaining a stronger insight into the differences between the present invention and the disclosures in the cited references.

Respectfully submitted,

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